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Label-free 3D super-resolution nanoscope with large field of view

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ABSTRACT

Photonic nanojet interferometry (PNI) permits three dimensional (3D) label-free and super-resolution surface characterization. PNI is based on coherence scanning interferometry (CSI), featuring Ångstrom level vertical resolution. Being an optical far-field technique, CSI is diffraction limited and according to the Abbe criteria, can laterally resolve, points that are separated by a few hundred nanometers. We overcame this limitation by using dielectric microspheres that generate photonic nanojets. Now sub 100 nm features can laterally be resolved while preserving the vertical resolution of the CSI system.

The microsphere material could be polymer or glass with a diameter between 8 and 12 µm, which limits the field of view (FoV) of the PNI system to ~10 μm^2. Here we present a method to increase the FoV of a PNI based device by stitching a sequence of adjacent 3D images.

We imaged a recordable Blu-ray Disc (BR-D) using a custom built Mirau type scanning white light interferometer with enhanced lateral resolution. Four 3D super-resolution images with constant 80% overlap, were stitched together using in-house software. The resulting high fidelity image shows that 45% overlap and the above described procedure could be used to enlarge the FoV of label-free 3D super-resolution imaging systems.

Keywords: Label-free 3D super-resolution, nanoscopy, stitching

1. INTRODUCTION

In 1873, Ernst Abbe described the resolution limit of an optical microscope in terms of objective numerical aperture (*NA*) and wavelength of used light. The limit of resolution in Abbe's theory is based on three assumptions: a) single objective lens, b) single photon absorption and emission in a time independent linear process at the same frequencies, c) and uniform illumination across the specimen with a wavelength in the visible range. By breaking these assumptions enhanced resolution in an experiment enhanced resolution in the optical microscope has been achieved [1].

1.1 Microsphere assisted super resolution

A new branch of label-free super-resolution microscopy emerged in 2004, when the discovery of the photonic nanojet (PNJ) - based resolution enhancement using high refractive index (RI), dielectric-microobjects was published [2]. The PNJ is a strongly focused beam of light generated on the shadow side of a micro object (e.g. cylinder, semi-sphere, sphere), when illuminated by a plane wave. The length of this focus is ~ 2λ (λ is the wavelength of the light used) at FWHM (Full Width at Half Maximum), while its width is ~ 0.5 ± 0.2λ at FWHM, depending on the parameters of the medium surrounding the micro object. The properties of PNJ depends on (a) the refractive index of the medium surrounding the micro objective, (b) the diameter of the focusing curvature of the microobject/diameter of the microsphere, (c) the refractive index of the microsphere and (d) the wavelength of the light. The mechanism of how

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these parameters influence the PNJ and how the PNJ enhances the resolution of the imaging, was studied extensively in the last decade, but still there is no common opinion on these topics [3, 4].

The first article considering two dimensional (2D) super-resolution was published 2010 and initiated the development of new devices exploiting microspheres of different sizes and materials [5]. Later, spheres with high RI embedded in polymers were used to enhance the resolution and, label-free resolution of $\lambda/7$ was achieved [6]. To enlarge the field of view (FoV) of 2D super-resolution devices PNJ generating systems involving fibers were introduced [7].

In 2016 the first publication concerning three dimensional (3D) appeared [8]. A Mirau type coherence scanning interferometer together featuring 11 µm melamine formaldehyde microspheres was used to image the surface of a Blue-ray Disc. The results showed better than 100 nm lateral and couple of nanometers axial resolution. Later a similar system, built in Linnik configuration, confirmed the obtained 3D super-resolution results [9].

1.2 3D stitching

One way to increase the FoV of an imaging system is to stitch sub-aperture images. *Stitching* is term used for enlarging the FoV by combining overlapping neighboring single scans with post-processing algorithms [10]. In contrast to *patching*, laying scanned images next to each other simply based on their position-offset coordinates, in stitching each single scan image is adjusted with algorithms to match the neighboring scans and hence to form an undistorted large area image. This way it is possible to maintain high magnification in each single scan and still cover a large sample.

When stitching images in a CSI system two main conditions should be fulfilled: (a) there must be a distance between the last objective lens and the sample to permit (b) lateral *xy*-scanning of the object. Today most of the commercial and custom developed interferometers are equipped with large area (e.g. 75 x 75 mm) electro-mechanical scanning systems. A system to guarantee a ca 1 µm gap between the sample and the microsphere is needed in PNI. For this we used 3D nano-positioning system to hold and position the PNJ generating microsphere.

2. METHODS

The FoV of a microsphere enhanced 2D or 3D super–resolution system is limited by the microsphere size. The FoV is approximately 30% of its diameter. To improve the imaging capabilities of our PNI we stitched several overlapping sub-aperture images. Our sample was a recordable Blu-ray Disc. The PNJ generating structure was a melamine formaldehyde microsphere with Ø= 11µm.

2.1 3D stitching algorithms

The sample movement between individual scans induce errors by offsetting the relative positions of the subimages. The movement is never perfect and errors against the desired position can be expected as shift, pan and tilt along all axes. Figure 1 illustrates the positioning errors caused by *xy*-movement between two individual scans. The figure shows misalignment errors dx, dy, dz and dθ between adjacent scans. These errors can be corrected by matching overlapping regions of neighboring scans to each other. Three general types of algorithms for 3D data images stitching are common [10, 11, 12]. Detailed explanation of the algorithms used in our custom build SWLI based system is discussed in [13].

2.2 PNJ enhanced SWLI

SWLI is non-contacting optical method that offers fast 3D profiling with Ångstrom level vertical (z) resolution [14]. Conventionally based on optical image acquisition with white light, laterally (xy) the resolution has been the same as in conventional microscopy [1]. SWLI based systems can be realized in three configurations – Mirau, Linnik, and Michelson. The first one is most used, because it is compact and allows a variety of magnifications - from 10x to 100x.

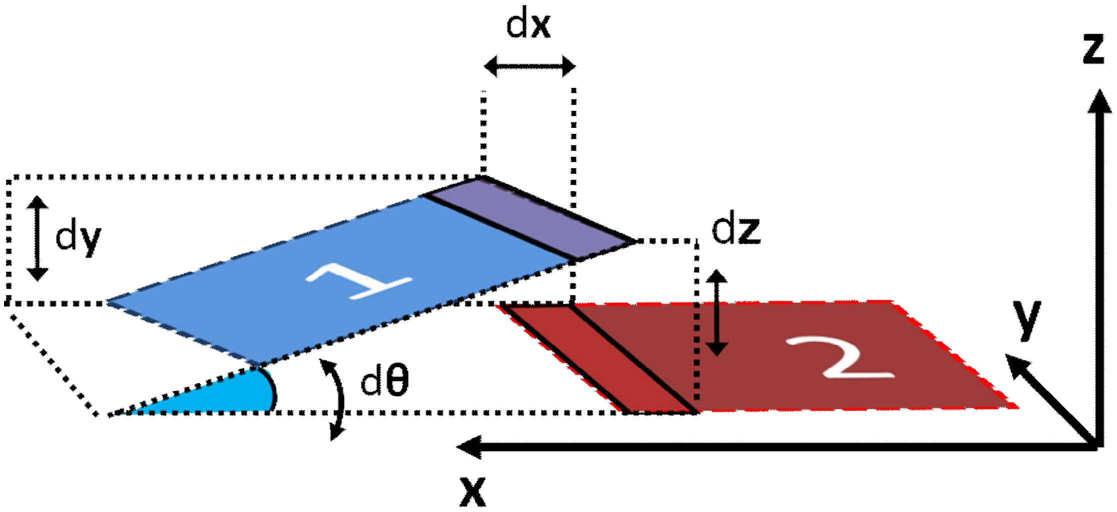


Figure 1. Positioning errors caused by movement between individual scans (1 and 2).

Recent research in optics and photonics has started to overcome the lateral resolution limit further [7, 13]. By introducing additional PNJ generating objects into the light path of the SWLI instrument, e.g. a micro-sphere, the xy-resolution has been improved to 100x100nm^2 [14, 15]. The conventional SWLI benefits remain: high z-resolution, fast scan time, and relatively large field of view within each single scan.

2.3 Setup

Our setup was based on halogen light illuminated (Pihlips 7724, 12V/100W) microscope frame (Nikon L-UEPI EPI) equipped with 50x Mirau-type interferometer objective (Nikon CF IC EPI Plan DI 50x, NA 0.55) attached to a piezo scanner (PI Pifoc P-721.CDQ). Images were recorded with a CMOS camera (Hamamatsu, Orca Flash 2.8). The sample was placed on a xy-translator stage (Standa 8MT167-25LS) bolted to a labjack (fig.2).

A melamine formaldehyde microsphere (Ø=11µm, Corpuscular, C-MFs-10.0) was held with vacuum at the tip of glass micropipette (WPI µTip, TIP2TW1). The pipette was attached to micromanipulator arm (SUSS Microtech PH100) to ensure the correct distance between the PNJ generating structure and the sample.

2.4 Sample

A recordable Blue-ray Disc (Verbatim BD-R Datalife 25gb 6x) features nanometer track dimensions. The BD-R was selected due to its tightly specified structure, polymer based materials, and good availability. It has a 320 nm pitch radial groove pattern that has 20-30 nm tall features with minimum groove size of 100 nm [16]. Such dimensions cannot be resolved with conventional optical white light microscopes [1].

The BD-R features multiple layers. The groove pattern is located on layer protected by thick top layer [17]. To achieve optically free access to this groove pattern the protective layer was peeled off. The peeling removes the top layer and leaves fresh groove pattern layer exposed for measurements. [18]. The BD-R disc was measured close to its center, a freshly prepared sample piece was used.

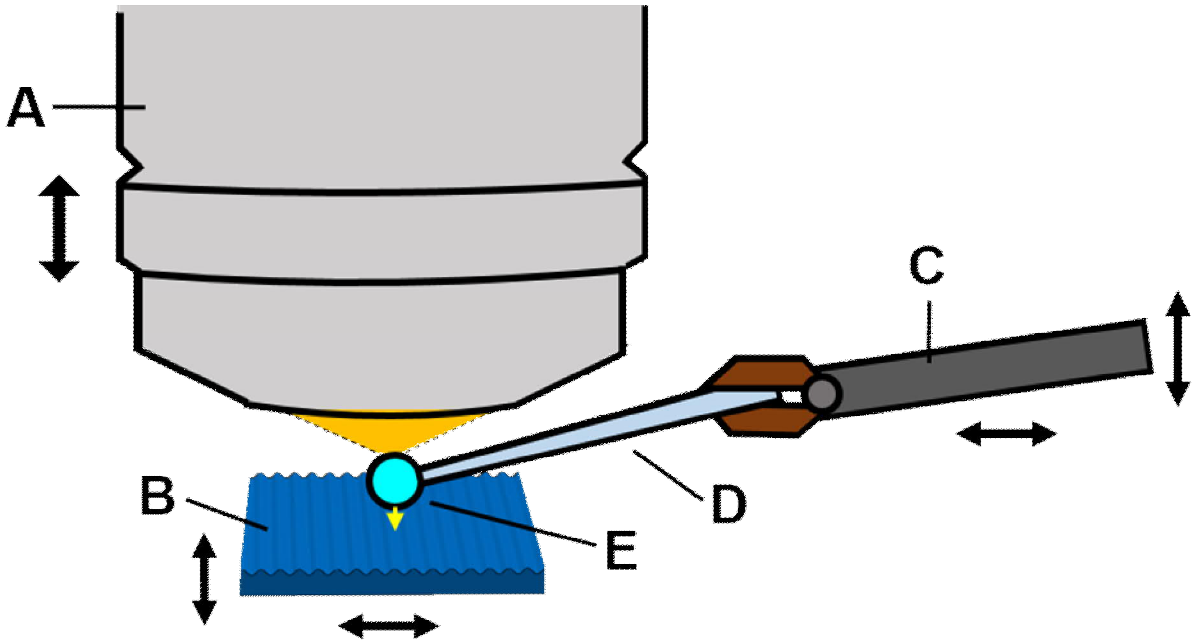


Figure 2. Setup: A) interferometric objective, B) BD-R sample, C) micromanipulator arm, D) glass micropipette, and E) microsphere.

A custom-made instrument software was used to control the scanning, data acquisition, and data analysis. Special attention was paid to aberration, which was removed after each scan. In our case the biggest error was introduced by defocusing caused by the microsphere being held between the Mirau objective and the sample. Additional software was used to ensure xy-scanning.

3. RESULTS

To check the PNI performance we did four sequential scans with 81% surface overlapping. First the microsphere was placed using the manipulator-pipette assembly close to the BD-R sample surface. In the next step the objective was focused through the microsphere to the interference image formed of BD-R sample surface. The 3D data was acquired by scanning the objective-to-microsphere distance across the image plane. Next a translation was done along one axis of the stage and the procedure was repeated. Every two adjacent scans were obtained with the translation between the scans being 0,4±0,1 µm.

Figure 3 shows individual images, with aberration removed and ready for stitching. Figure 4 shows the stitched images and the reconstructed profiles. To study the necessary % of overlap we first stitched all images (fig. 4a), after that first and third image (fig. 4b) and finally only the first and fourth image (fig. 4c). The profiles reconstructed in the area of the individual scan and in the overlapping areas are shown. The stitched images maintained the lateral and vertical resolution of the individual images. The FoV of the PNI system was increased.

The overlap between first and third scan is 62% whereas between the first and fourth scan it is 45%. This appears to be close to what is needed to avoid degrading the image.

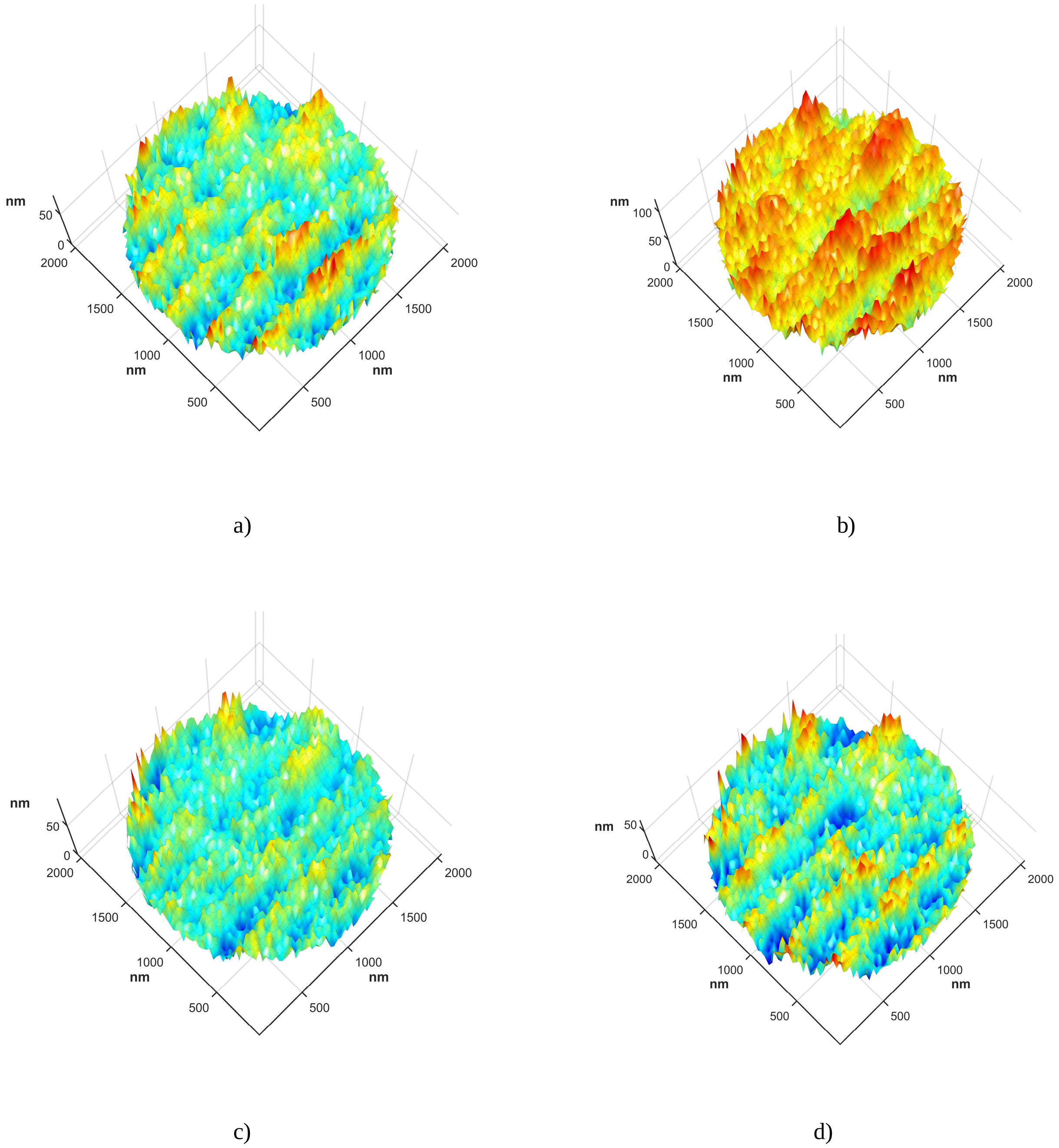
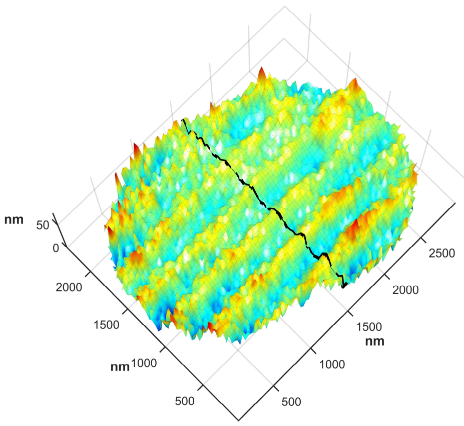
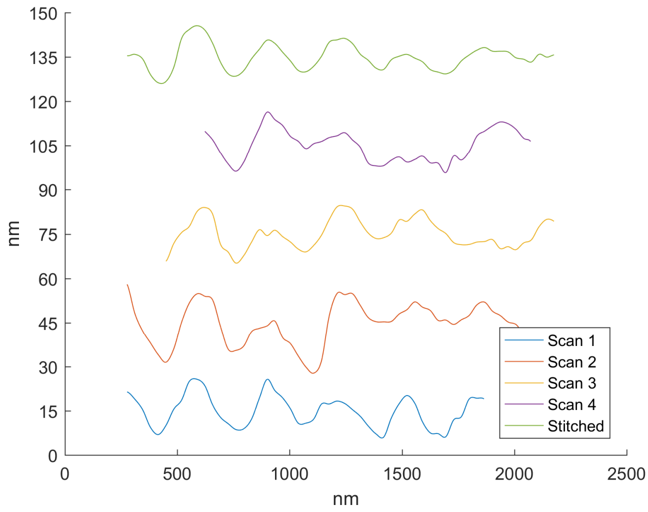
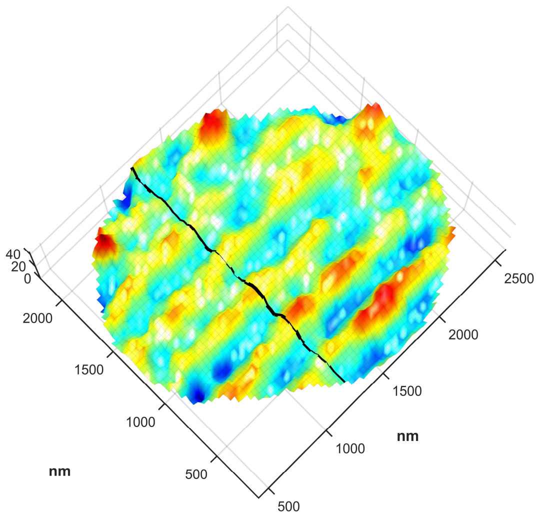


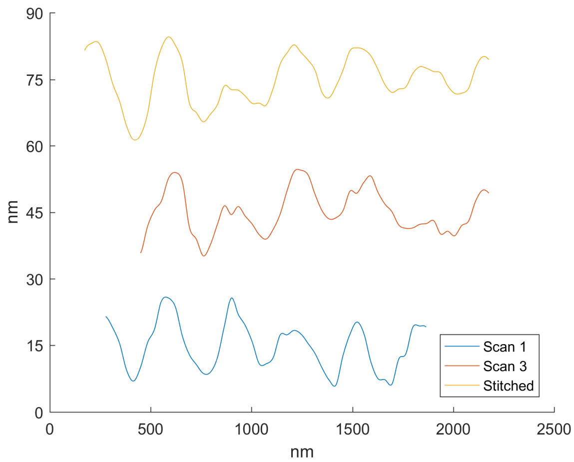
Figure 3. Four individual images of the surface of the BR-D taken consecutively with aberration removed and ready for stitching: a) image of the first scan, b) image of the second scan, c) image of the third scan and d) image of the fourth scan.



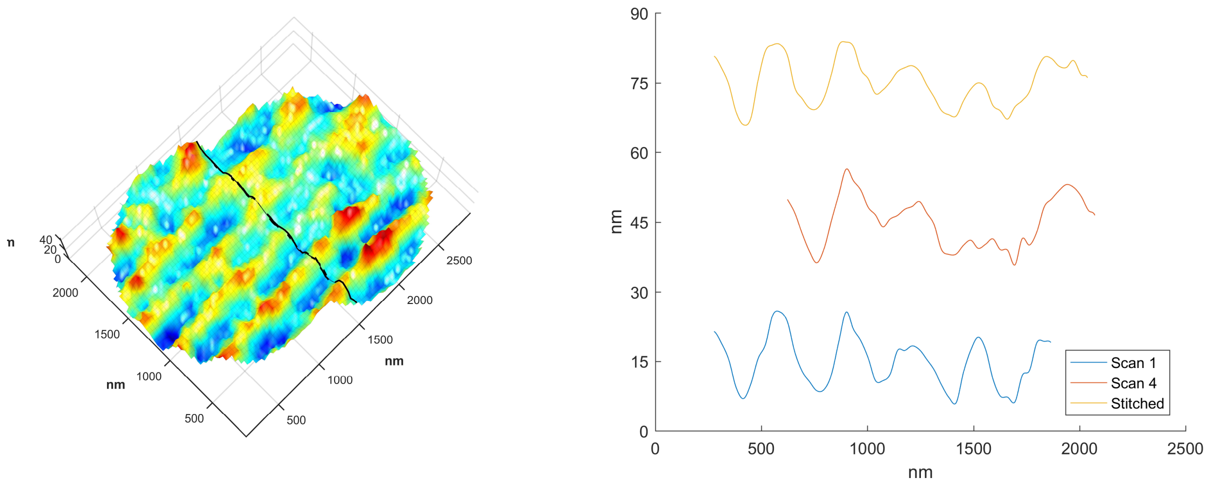


a) All images stitched.





b) First and third images stitched.



c) First and fourth images stitched.

Figure 4. Stitched image from four and two scans of Blu-ray Disk surface showing nm-scaled topological features. The black line illustrates the position of line extraction presented in the graph on right side. The lines in this graph represent surface data extracted from individual scans separately and from the stitched surface data.

4. CONCLUSIONS

A method to increase the FoV of a PNI device, based on stitching together a sequence adjacent 3D images was demonstrated. We imaged a recordable Blu-ray Disc using a custom-built Mirau-type PNI. Four 3D super-resolution images with 80% overlap were stitched together using in house developed software. The resulting image maintained the accuracy of the individual images if the overlapping area was 45%.

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